

Sediment-based carbon nutrition in tropical alpine *Isoetes*

JON E. KEELEY, DARLEEN A. DEMASON,
RENEE GONZALEZ and KENNETH R. MARKHAM

Introduction

Isoetes (Isoetaceae) is a genus of small herbaceous plants often aligned with *Lycopodium* and *Selaginella*. There are more than 150 species distributed worldwide, typically in aquatic habitats (Tryon & Tryon 1982). A particularly intriguing aspect of the physiology of these plants is the presence of Crassulacean Acid Metabolism (CAM) (Keeley 1981, 1982), a photosynthetic pathway commonly associated with terrestrial xerophytes. CAM was selected for in these species by the daytime carbon limitation characteristic of their oligotrophic aquatic habitats (Keeley & Busch 1984; Boston & Adams 1985).

Across its range, *Isoetes* has radiated into a variety of aquatic as well as some terrestrial habitats and these environments have selected for a number of different structural-functional syndromes (Keeley 1987). Aquatic species occur in lacustrine habitats where they are permanently submerged throughout their life cycle and in amphibious environments where they alternate seasonally between aquatic and terrestrial conditions. In general, all aquatic species so far tested possess a well-developed CAM pathway while under water but lose this pathway when grown in an aerial environment. True terrestrial species of *Isoetes* are few in number although such species are known from most parts of the world. They readily fall into one of two groups: vernaly active, summer-deciduous species at relatively low elevations in temperate latitudes; and evergreen species restricted to very high elevations (> 3500 m) in tropical latitudes. The former species show no CAM activity (even if artificially submerged), possess stomata and presumably depend entirely on C₃ photosynthesis. The tropical alpine species have variable levels of CAM metabolism but are unique in the terrestrial vascular plant kingdom in their complete lack

of stomata. There is some evidence (Keeley *et al.* 1984) that these taxa rely upon the sediment for their source of photosynthetic carbon.

Sediment-based carbon nutrition in the terrestrial *Isoetes* (*Stylites*) *andicola*

Dependence upon the sediment for carbon acquisition is known in aquatic plant species, particularly those with the 'isoetoid' growth form (e.g. Wium-Andersen 1971; Sondergaard and Sand-Jensen 1979; Richardson *et al.* 1984; Boston *et al.* 1987). These species are restricted to oligotrophic lakes where the very low inorganic carbon levels in the water, coupled with the very high diffusive resistance of water, limit carbon availability. In these environments the much higher carbon levels in the sediment put a premium on carbon acquisition through the roots.

On land the photosynthetic organs of higher plants normally have access to atmospheric CO₂ through stomatal pores and the dependence upon the sediment for carbon acquisition was unknown until it was reported for *Isoetes andicola* (Keeley *et al.* 1984).

Field work in the Peruvian Andes by Rauh and Falk in the 1950s brought to light a relatively unusual taxon in the Isoetaceae (although the species was collected by earlier botanists, Asplund No. 11830, 1940, STC). Amstutz (1957) named this *Stylites andicola*, separating it from the well-known genus *Isoetes* by the terrestrial habitat, elongated and branching stem with unbranched roots initiated along one side of the stem and sporangia elevated above the sporophyll base. Rauh & Falk (1959) provided further reason for separation by reporting a chromosome number of $2n = c. 58$, a number quite distinct from the $2n = 22$, or multiples thereof, typical of the genus *Isoetes*. Further collections of Andean *Isoetes*, however, have revealed taxa with many of the traits thought to be unique to *Stylites* (Kubitzki & Borchert 1964; Gomez 1980; Karrfalt & Hunter 1980; Fuchs-Eckert 1982; Hickey 1985) and recent counts of *Stylites* have given a chromosome number of $2n = 44$ (Hickey 1984; L. D. Gomez and J. Keeley, unpublished data). Thus, there are good reasons for including *Stylites* within *Isoetes* and thus the correct name for 'stylites' is *Isoetes andicola* (Amstutz) Gomez.

Isoetes andicola is endemic to several widely scattered populations mostly above 4000 m in central and southern Peru. Reports by Feuerer that the species 'is quite common' in west central Bolivia (Hickey 1985) are in error. Thorough examination of Bolivian sites described by Feuerer (personal communication) uncovered no *I. andicola* (J. Keeley,

personal observations); possibly the similar appearing *Plantago rigida*, which was quite abundant at these sites, was mistaken for *I. andicola*. The report of *I. andicola* from Colombia (Cleef 1981) is also based on a misidentified specimen (J. Keeley, personal observation). The habitat of *I. andicola* is not well documented but, based on reports in the literature, personal observations and data on herbarium specimens, it appears to be largely restricted to sites around the outer edges of bogs or lakes. At most sites it is distributed at an elevation of c. 1 m above the surface level of the standing water. Such microhabitats would be saturated during the wet season but potentially very xeric during the dry season (e.g. Table 9.1).

This species forms small rosettes from the apices of elongated, often branching, stems (Figure 9.1). It is unusual among *Isoetes*, both terrestrial and aquatic, in the extent of biomass underground. Over half of the biomass is tied up in roots and only a fraction of the leaf material is

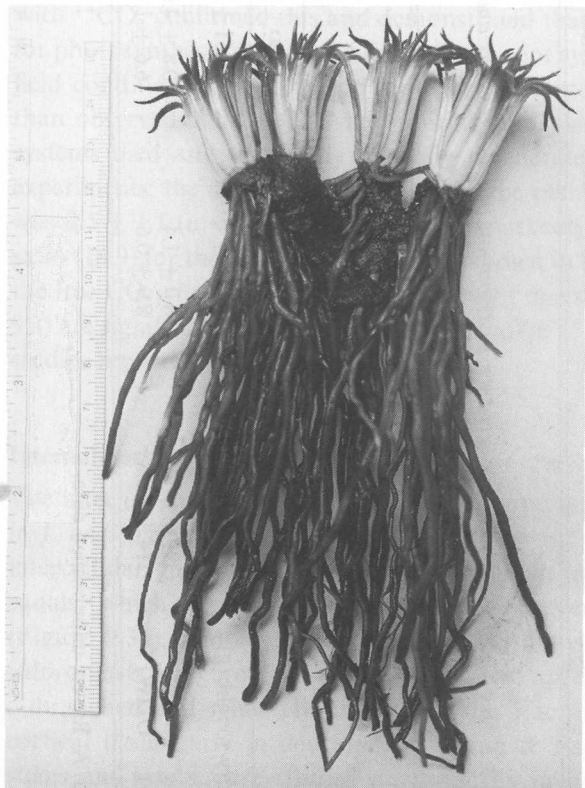


Figure 9.1. *Isoetes andicola*, 'stylites', collected near Junin, Peru; scale is a 15 cm ruler.

Table 9.1 Climatic data for La Oroya, Departamento Junin, Peru (11° 31' S, 75° 56' W), a station at 3712 m within 50 km of several Isoetes andicola populations

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Precipitation (mm)	61	71	75	31	3	1	22	9	61	36	44	4.0
Temperature (°C)												
Mean minimum	2.9	3.9	3.4	3.1	0.1	-0.7	0.9	0.9	3.3	3.7	3.7	2.0
Mean maximum	16.5	16.0	18.2	17.5	18.0	17.0	15.8	16.8	16.0	19.1	19.0	17.0

Table 9.2 Dry weight, carbon, and nitrogen distribution in leaves, stems and roots of Isoetes andicola collected near Junin, Peru (n = 4)

	Leaves				Stems		Roots	
	Green portions		White portions		Stems		Roots	
	$\bar{X} \pm \text{SD}$		$\bar{X} \pm \text{SD}$		$\bar{X} \pm \text{SD}$		$\bar{X} \pm \text{SD}$	
Oven dry weight (g)	0.055 ± 0.011		0.148 ± 0.027		0.361 ± 0.032		0.821 ± 0.086	
%	4.0		10.7		26.0		59.3	
C (mg g ⁻¹ ODW)	399.0 ± 1.9		398.0 ± 1.4		420.8 ± 4.0		413.3 ± 4.1	
N (mg g ⁻¹ ODW)	14.8 ± 0.8		8.0 ± 0.6		16.6 ± 1.2		8.0 ± 0.5	

From J. A. Raven, unpublished data.

chlorophyllous and occurs aboveground (Table 9.2). Although there is no significant difference between the different parts in terms of C per unit of dry weight, the green leaves have significantly more N than underground achlorophyllous parts of leaves or roots (Table 9.2). Only the tips of the leaves are chlorophyllous (4% of total biomass) and these barely emerge from the sediment (Figure 9.2). Roots extend to at least 50 cm depth, although 80% occur in the upper 20 cm.

Structurally, the leaves of *Isoetes andicola* are unusual among terrestrial vascular plants in the lack of stomata. Additionally, these leaves are covered by a very thick cuticle. These observations led to the initial investigation of its unique physiology (Keeley *et al.* 1984), where it was demonstrated that there is very little CO₂ conductance across the leaf epidermis; dual isotope porometer measurements gave CO₂ conductances of 0.001–0.005 mm s⁻¹ (values nearly an order of magnitude lower than those of desert cacti with closed stomata: Kluge & Ting 1978). Studies with ¹⁴CO₂ confirmed this and demonstrated that the bulk of the carbon for photosynthesis is obtained through the root system (Table 9.3). Under field conditions the CO₂ captured by roots is potentially much greater than observed in Table 9.3 since, during transplanting, the original root systems died and were only partially regenerated at the time of these experiments; the root/green-leaf ratio for the plants sampled in Table 9.3 was 2.5 ± 1.1 ($n = 12$), which contrasts markedly with the much higher ratio (14.9) for the field-collected plants shown in Table 9.2. Additionally, the free CO₂ concentrations in the sediment measured in the field (Table 9.4) are significantly higher than the 0.78 mol m⁻³ used for the laboratory studies reported in Table 9.3.

Internal pathway of carbon uptake in *Isoetes andicola*

The internal route taken by carbon moving from the roots to the leaves in *I. andicola* is unknown, although the case for CO₂ diffusion through intercellular gas space is strong. The leaves have four large lacunal air canals, which occupy more than half of the cross-sectional airspace (Figure 9.3a), and are surrounded by mesophyll cells with abundant chloroplasts (Figure 9.3b). The roots are up to 3 mm in diameter, unbranched and relatively hollow (Figure 9.3c), due to the collapse of cortical tissue early in development (Rauh & Faulk 1959). The leaves, stems and roots were studied anatomically in order to obtain precise measures of the volume of airspace along the root–stem–leaf pathway. These organs were fixed in FAA, dehydrated through an alcohol series



Figure 9.2. *In situ* view of *Isoetes andicicola* (I.a.) and associated species *Plantago rigida* (P.r.) at Junin, Peru. Diameter of the coin, c. 2.8 cm.

and embedded in paraplast. Sections were photographed and the extent of intercellular airspace was measured with Sigma Scan Measurement System software (Jardel Scientific, Sausalito, CA). Percentage of cross-sections occupied by airspace ($\bar{X} \pm \text{SD}$, $n = 10$) was: leaves = $58.0\% \pm 7.9$, stems = $35.1\% \pm 11.4$, roots = $75.2\% \pm 4.8$.

If carbon dioxide diffuses from the sediment to the photosynthetic leaf

tips through intercellular airspace, we can calculate whether or not the flux of CO₂ along this pathway is sufficient to maintain observed rates of photosynthesis (Table 9.3). Here we assume that CO₂ in the gas phase of the root is in equilibrium with free CO₂ in the soil solution and that a single root supplies CO₂ to a single leaf (a close approximation in light

Table 9.3 ¹⁴CO₂ uptake in the light and dark by chlorophyllous portions of *Isoetes andicola* leaves grown under moist or wet conditions

		CO ₂ uptake by green leaves (μmol CO ₂ mg ⁻¹ chl h ⁻¹)	
		¹⁴ CO ₂ fed to leaves	¹⁴ CO ₂ fed to roots
		$\bar{X} \pm \text{SD} (n)$	$\bar{X} \pm \text{SD} (n)$
Light	(Moist)	0.74 + 0.14 (3)	39.75 + 6.14 (3)
	(Wet)	1.12 + 1.05 (2)	18.78 + 0.79 (2)
Dark	(Moist)	0.24 + 0.16 (2)	0.51 + 0.07 (2)

From Keeley *et al.* 1984.

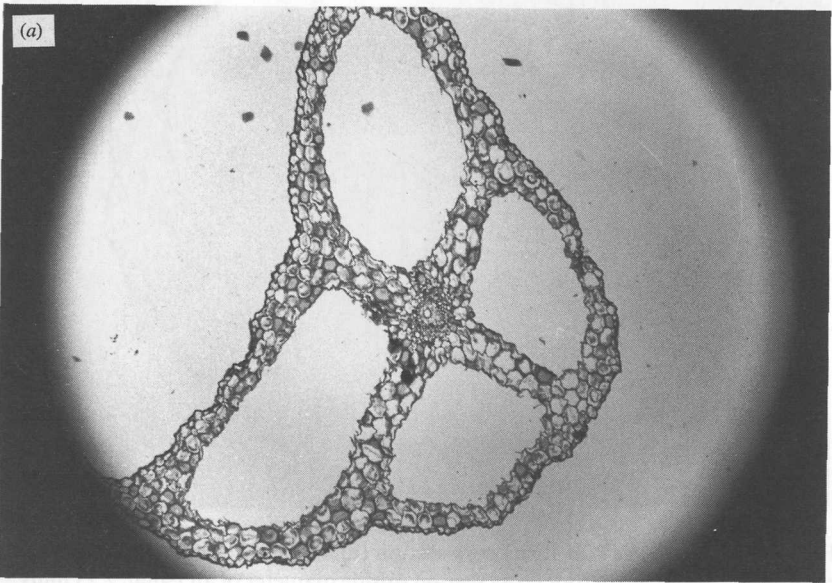


Figure 9.3. Cross-sections of *Isoetes andicola*. (a) Whole leaf (dimensions of largest lacunae, 0.93 × 0.40 mm. See over for Fig. 9.3(b) and (c)

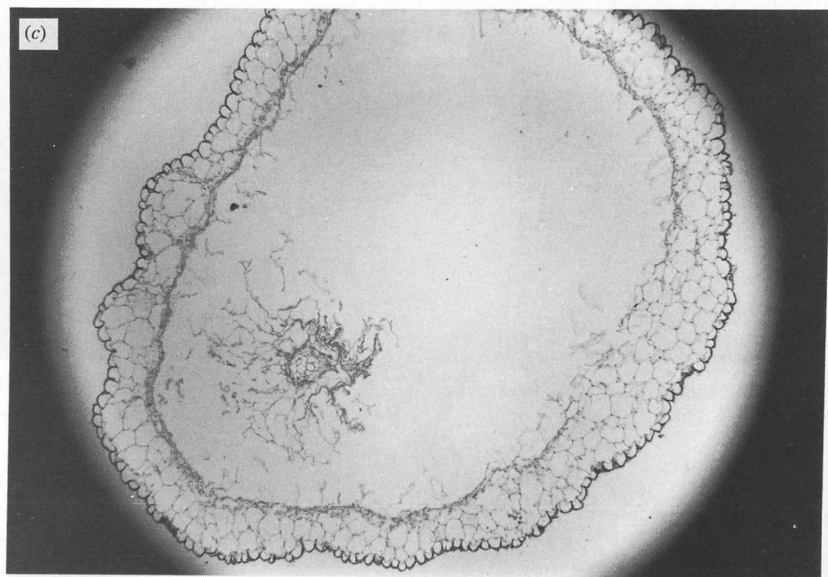
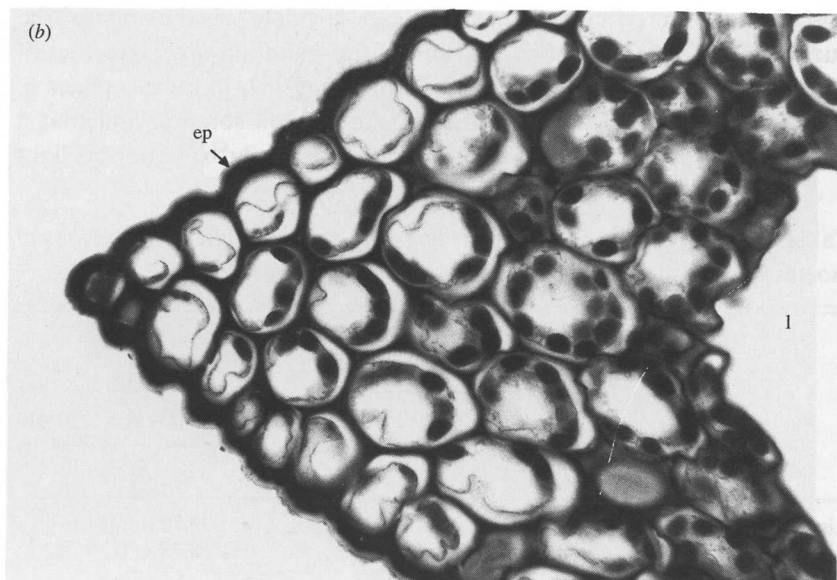


Figure 9.3. (*continued*) (b) Leaf cross-section (diameter of largest cell, 80 μm); ep, epidermis; l, lacunae; (c) root cross section (inside diameter of air canal, 2.25 mm).

Table 9.4 *Water chemistry characteristics of interstitial sediment water from sites with terrestrial Isoetes species*

Species	Site	Date	(n)	pH	Free CO ₂ (mol m ⁻³)	Oxygen (mol m ⁻³)
<i>Isoetes andicola</i>	Junin, Peru, 4200 m	Nov	(3)	5.95–6.25	3.59–4.93	0.02–0
<i>Isoetes novo-granadensis</i>	Quito–Baeza Pass, Ecuador, 4050 m	Nov	(3)	5.60–6.00	1.46–1.77	0.16–0
		Jul	(1)	5.77	3.63	
<i>Isoetes andina</i>	L. Chisaca, Colombia, 3650 m	Dec	(4)	4.44–4.80	2.15–7.26	0.10–0

See Appendix 9.1 for precise localities, and Keeley & Busch 1984 for methods.
From J. E. Keeley, unpublished data.

Table 9.5 Calculations of carbon dioxide diffusion through intercellular airspace of roots, stems and leaves of *Isoetes andicola* based on the lowest sediment carbon dioxide concentration measured in the field (Table 9.4) and a CO_2 diffusion coefficient at 4000 m (0.063 MPa) and 10°C

$$\text{Flux of } \text{CO}_2 = \frac{[\text{CO}_2]_{\text{sediment}} - [\text{CO}_2]_{\text{leaf}}}{r_{\text{leaf}} + r_{\text{corm}} + r_{\text{root}}}$$

where resistance, $r = \frac{\text{Length of pathway}}{\text{Diffusion coefficient of } \text{CO}_2} \times \frac{1}{\text{Cross-sectional airspace}}$

$$r_{\text{leaf}} = \frac{3 \times 10^{-2} \text{ m}}{2.27 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}} \times \frac{1}{1.39 \times 10^{-6} \text{ m}^2} = 0.95 \times 10^9 \text{ s m}^{-3}$$

$$r_{\text{corm}} = \frac{1 \times 10^{-2} \text{ m}}{2.72 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}} \times \frac{1}{30.38 \times 10^{-6} \text{ m}^2} = 1.44 \times 10^7 \text{ s m}^{-3}$$

$$r_{\text{root}} = \frac{7 \times 10^{-2} \text{ m}}{2.72 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}} \times \frac{1}{2.13 \times 10^{-6} \text{ m}^2} = 1.37 \times 10^9 \text{ s m}^{-3}$$

$$\text{Flux of } \text{CO}_2 = \frac{3.589 \text{ mol m}^{-3} - 5 \times 10^{-4} \text{ mol m}^{-3}}{(0.95 \times 10^9 + 1.44 \times 10^7 + 1.37 \times 10^9) \text{ s m}^{-3}}$$

$$= 1.5372 \times 10^{-9} \text{ mol s}^{-1} [\text{leaf}^{-1}]$$

$[1.3 \times 10^{-2} \text{ g FW green tissue leaf}^{-1} \text{ and } 1.00 \text{ mg Chl g}^{-1} \text{ green tissue}]$

$$\text{Flux of } \text{CO}_2 = 425 \mu\text{mol } \text{CO}_2 \text{ mg}^{-1} \text{ Chl h}^{-1}$$

of the plant architecture: Figure 9.1). According to Fick's Law of gas diffusion, flux of CO_2 would be a function of the concentration gradient between the root and leaf and the internal resistances along the root–stem–leaf pathway (Nobel 1983). The calculations shown in Table 9.5 utilized the lowest $[\text{CO}_2]$ measured in the field (Table 9.4) for the source, a value of $5 \times 10^{-4} \text{ mol m}^{-3}$ as a reasonable estimate for the $[\text{CO}_2]$ at the site of photosynthesis, and a diffusion coefficient for CO_2 at 4000 m and 10°C . Resistances were calculated for a leaf, stem, and root of average length and diameter and thus, for an average leaf we calculate CO_2 diffusion from the sediment of $1.5372 \times 10^{-9} \text{ mol s}^{-1}$. Using a chlorophyll concentration of 1 mg g^{-1} fresh weight of chlorophyllous tissue (Keeley *et al.* 1984) and a value of 0.049 g fresh weight per leaf ($n = 10$), of which only 27% is chlorophyllous (Table 9.2), we calculate the flux of CO_2 as $425 \mu\text{mol } \text{CO}_2 \text{ mg}^{-1} \text{ Chl h}^{-1}$.

This is perhaps a conservative estimate of the rate of CO_2 diffusion from the sediment to the leaves under field conditions. Calculations used the lowest CO_2 source concentration measured in the field and a pathway from roots through 1 cm of stem was considered, even though roots are initiated acropetally so that newly initiated roots are connected almost directly to leaves. Even so, it is more than sufficient to account for the maximum photosynthetic rate of $39 \mu\text{mol mg}^{-1} \text{Chl h}^{-1}$ shown in Table 9.3. However, Table 9.3 experiments were performed with a CO_2 concentration of only 0.78 mol m^{-3} in the solution bathing the roots. Using this value for the 'source', and a diffusion coefficient at 20°C and 0.1013 MPa , we calculate $59 \mu\text{mol mg}^{-1} \text{Chl h}^{-1}$, which still could account for rates of photosynthesis measured in the laboratory (Table 9.3). Other factors not considered include the diaphragms that are spaced several millimeters apart in the leaf lacunae. Because they are perforated and very thin, though, the resistances contributed by them are insignificant when added to the resistances already considered. One oversimplification is the assumption that one root contributes carbon to one leaf, but in reality the number of leaves is typically greater than the number of roots. The calculations presented here, however, suggest that a single root is likely to be able to supply CO_2 at a rate sufficient to saturate photosynthesis in more than a single leaf.

Other mechanisms of gas movement, such as mass flow as observed by Dacey (1980) in *Nuphar*, may be operating, although it is not clear how such a system would work in this case. Fixation of CO_2 in the roots and movement through the vascular system is possible as phosphoenolpyruvate (PEP) carboxylase activity is present in the roots (J. E. Keeley, unpublished data). However, PEP carboxylase levels are many times lower in roots than in leaves (on a fresh weight basis) and the vascular cylinder represents a very minor part of the total root volume. Also, the very small vascular cylinder would make the hydration of carbon dioxide to bicarbonate, and movement in solution to the leaves, an unlikely hypothesis. In light of the discussion above, diffusion of CO_2 through internal airspace would seem to be the most parsimonious model of carbon movement from the sediment to the leaves of *Isoetes andicola* (stylites).

CAM in *Isoetes andicola*

Another aspect of the physiology of these plants is the presence of Crassulacean Acid Metabolism (CAM). Under natural conditions the chlorophyllous portions of leaves showed a diurnal change in malic acid

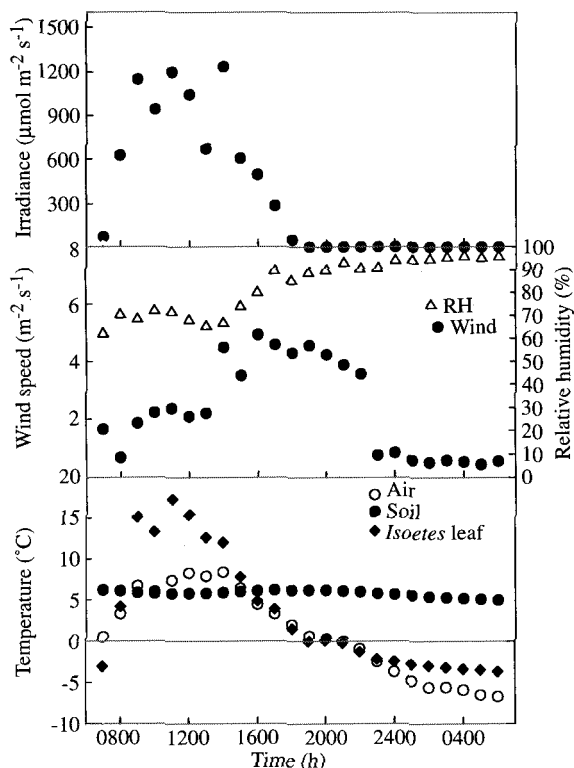


Figure 9.4. Microclimate and leaf temperatures for *Isoetes andicola* at the Junin site, Peru (November 1985). See Appendix 9.1 for exact location and Keeley & Keeley (1989) for methods.

of $40\text{--}46\ \mu\text{mol g}^{-1}$ fresh weight in both summer and winter (Appendix 9.1). Due to the lack of gas exchange between leaves and the atmosphere, CAM undoubtedly was not selected for the same reason as in other terrestrial CAM plants. Potential functions of CAM would be: (i) to recycle dark-respired CO_2 ; (ii) to allow carbon fixation to proceed over the entire 24-hour period, thus effectively doubling the time for carbon fixation; (iii) daytime decarboxylation of malic acid in the CAM pathway may reduce photoinhibition, a particular concern for high elevation plants (Mooney *et al.* 1974).

Overnight carbon fixation is particularly noteworthy in this tropical alpine environment during the winter months when air temperatures routinely drop below freezing. Microclimatic data for one such night are shown in Figure 9.4. Despite the fact that leaf temperatures dropped to

below 0 °C by 2100 h, this plant was still able to accumulate a substantial concentration of malic acid (Appendix 9.1). CAM functioning at near or below zero temperatures has also been observed in tropical alpine cacti (Keeley & Keeley 1989).

In summary, the bulk of the carbon gain in *I. andicola* is obtained from the sediment. Most of this uptake occurs during the day and it is presumably fixed via the (C₃) PCR (pentose carbon reduction) cycle reactions; ribulose biphosphate carboxylase levels are sufficient (70–134 $\mu\text{mol mg}^{-1} \text{ Chl h}^{-1}$) to account for observed light fixation rates. Overnight, CO₂ uptake continues at a low level and some of the respiratory carbon is recycled, both presumably through CAM-like carbon fixation; PEP carboxylase levels are sufficient to account for levels of acid accumulation (24–34 $\mu\text{mol mg}^{-1} \text{ Chl h}^{-1}$) and dark-fixed CO₂ has been shown to be incorporated into malic acid. Since relatively little gas exchange occurs across the leaf epidermis, the photosynthetic oxygen generation is likely to result in a substantial concentration gradient between the leaf and the sediment. Outward diffusion of oxygen from the roots probably contributes to the prevention of anaerobic conditions in the sediment (Table 9.4) and to enhancing conditions for mineralization of the organic matter in the sediment (e.g. Sorrell & Dromgoole 1987).

Carbon isotopes in *Isoetes andicola*

Independent confirmation of these conclusions has recently been provided by carbon and hydrogen isotope data. Sternberg *et al.* (1985) found that the $\delta^{14}\text{C}$ of the peat *Isoetes andicola* was growing in (at the Junin site described in Keeley *et al.* 1984) was +36‰ (relative to the normal standard), a value far below the atmospheric level typical of the post-nuclear bomb testing era, i.e. post-1960 (Table 9.6). Based on this they contend that the peat was formed at some time during the mid-1950s and, since *I. andicola* cannot be older than its substrate, the formation of its biomass began at the earliest in the mid-1950s continuing up to December 1982, the date of collection. For comparison, Table 9.6 shows $\delta^{14}\text{C}$ values for several tree rings that grew between 1960 and 1970 indicating that large amounts of bomb-produced carbon-14 were incorporated into the tree biomass. Sternberg *et al.* (1985) calculated that a plant growing continuously from 1955 to 1982, fixing only atmospheric CO₂, should have a $\delta^{14}\text{C}$ value of about +370‰. Since the $\delta^{14}\text{C}$ values of the atmosphere between 1955 and 1982 were as high as or higher than +235‰ (the atmospheric level in 1982), any plant that started growth after 1955

Table 9.6 Isotope abundances in *Isoetes andicola*, the peat it was growing in and associated species, *Plantago rigida* and *Distichia muscoides*, all collected in December 1982 from the Junin, Peru site (see Appendix 9.1). For comparison are the isotope abundances in atmospheric CO₂ and growth rings in spruce (*Picea* sp.) from Oregon, USA (from Sternberg et al. 1985)

	$\delta^{14}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)	$\delta^{13}\text{C}$ (‰)	δD (‰)
Peat	+36		-26.6	
<i>Isoetes andicola</i>	+142	+18.6	-22.5	-47
<i>Plantago rigida</i>		+19.6	-24.5	-83
<i>Distichia muscoides</i>		+19.1	-22.2	-103
Atmospheric CO ₂ (December 1982)	+235		-7.0	
Growth rings from spruce				
1960	+250			
1965	+900			
1970	+472			

and continued to grow until 1982, fixing only atmospheric CO₂, would have a $\delta^{14}\text{C}$ value $\geq +235\text{‰}$. The $\delta^{14}\text{C}$ value of *I. andicola* biomass was only +142‰ (Table 9.6), which is much less than what would be observed for a plant fixing atmospheric carbon dioxide since 1955, or any time after 1955, until 1982. Therefore, this plant must have obtained a large portion of its carbon by fixing CO₂ derived from the ¹⁴C-depleted decomposing peat, and this must have been derived directly from the soil atmosphere, since the CO₂ evolved from the sediment would be quickly mixed with the atmosphere on these open windy sites.

Although stable carbon isotope values are often used to distinguish photosynthetic modes in terrestrial plants (e.g. ratio ¹³C/¹²C), they do not readily distinguish *I. andicola* from associated non-CAM species (Keeley et al. 1984; Table 9.6; Appendix 9.1). One important reason for this is that the source of carbon for CAM is decomposing peat, which reflects previous fractionation events during the lifetime of the plants making up the peat. Other factors that could affect the $\delta^{13}\text{C}$ ratio of *I. andicola* are the very different diffusional resistances not encountered by normal terrestrial CAM plants. These factors could also account for the related observation that aquatic *Isoetes* species with CAM have $\delta^{13}\text{C}$ values indistinguishable from non-CAM aquatic plants with which they coexist (Keeley et al. 1987).

Hydrogen isotope ratios, on the other hand, readily distinguish CAM from non-CAM plants in both aquatic (Sternberg et al. 1984) as well as

in terrestrial species (Ziegler *et al.* 1976; Sternberg & DeNiro 1983); in both environments CAM species are enriched with deuterium relative to associated non-CAM species. The δD value for *I. andicola* was 36–56‰ higher than the δD values for associated non-CAM species (Table 9.6) and this is consistent with the report of CAM for *I. andicola* (Appendix 9.1).

Characteristics of plants associated with *Isoetes andicola*

Isoetes andicola populations typically consist of hundreds of rosettes interspersed amongst other vascular plants. A strong degree of convergence is evident in the superficially similar rosette character of many of these other species e.g. *Plantago rigida* H.B.K. (Figure 9.2) and *Distichia muscoides*. Despite the marked morphological similarity with *I. andicola*, it seems likely that these species obtain the bulk of their carbon from the aerial atmosphere; their leaves have abundant stomata and a dense chlorophyllous tissue beneath the upper epidermis (e.g. Figure 9.5). Also, their leaves are almost entirely aboveground and chlorophyllous, in contrast to *Isoetes* where only the upper quarter of the leaf is above ground

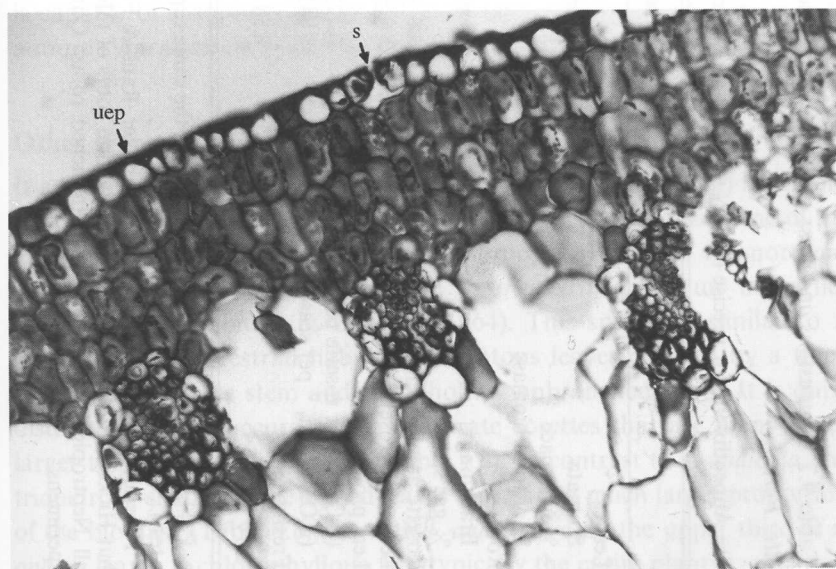


Figure 9.5. *Distichia muscoides* leaf cross-section (diameter of epidermal cell, 40 μm); uep, upper epidermis; s, stoma. Stomatal density estimated at 38 000 cm^{-2} .

Table 9.7 Dimensions and mass of the terrestrial Isoetes and associated rosette-forming species from the Junin site, Peru

See Appendix 9.1 for precise locality. Methods are described in the text.

	<i>Isoetes andicola</i>	<i>Distichia muscoides</i>	<i>Plantago rigida</i>
Leaves			
Stomata	Absent	Abundant	Abundant
% gas space	58	17	8
% of total biomass	24	52	33
Roots			
Branching	Absent ^b	Extensive	Extensive
% gas space	75	18	35
Rosette areal coverage (cm ² per rosette) ^a	12.0 ± 6.7	1.3 ± 0.7	8.4 ± 3.6
Leaves per rosette	78.9 ± 28.7	23.1 ± 6.3	16.5 ± 2.1
Leaf production			
(g ODW per rosette per season)	0.473 ± 0.280	0.125 ^a ± 0.043	0.100 ^a ± 0.048
(g ODW cm ⁻² per season)	0.041 ± 0.021	0.096 ± 0.054	0.012 ± 0.006
Fresh weight	8.88	9.28	3.93
Dry weight			

^a $\bar{X} \pm \text{SD}$, $n = 10$ (values within a row with the same superscript are not significantly different at $p > 0.05$, with 2-tailed t -test).

^b Possibly there is plasticity in this character. Rauh & Falk (1959) stated that the roots of *I. andicola* were unbranched, but Hickey (1985) noted that in cultivation roots of this species were dichotomously branched and he concluded that all Neotropical *Isoetes* produced branched roots. Our designations here are based on hundreds of field-collected specimens.

and chlorophyllous (Table 9.2). Thus, aboveground chlorophyllous tissue in *Isoetes* represents only 4–6% of the total biomass, whereas a third to a half of the biomass, in *Plantago* and *Distichia*, respectively, is in the leaves (Table 9.7) and this is roughly comparable to the aboveground chlorophyllous tissue. Not only is much less of the biomass belowground, these taxa have root systems markedly unlike *Isoetes andicola*; there is a proliferation of secondary branches, in marked contrast to the complete unbranched character of *I. andicola* roots. *Plantago* and *Distichia* also have relatively little intercellular gas space in leaves and roots (Table 9.7) suggesting less potential for CO₂ diffusion from the sediment. There is no indication of CAM in *Plantago* and *Distichia* (Appendix 9.1).

Seasonal biomass production of leaves for these three species was compared by marking leaves near the beginning of the growing season (November) and then harvesting all new leaves at the end of the growing season in July. Total biomass production was significantly greater for *Isoetes* rosettes (Table 9.7). However, this is a function of the larger size of their rosettes, as there was no significant difference in leaf biomass production per cm² of rosette surface area. At any rate, it is somewhat surprising that an astomatous species, cut off from CO₂ exchange with the atmosphere, and with some level of CAM-like dark fixation of carbon, is capable of sustaining leaf production rates comparable to plants with a more typical mode of carbon uptake.

Other tropical Alpine terrestrial *Isoetes*

It appears that the syndrome described for stylites (*I. andicola*) is present to some extent in other tropical alpine terrestrial *Isoetes* taxa. One of the most widespread of these is *I. andina* Spruce ex Hook. of the northern Andean páramo (synonymous with *I. triquetra* A. Braun of earlier literature, e.g. Kubitzki & Borchert 1964). This species is similar to *I. andicola* in its terrestrial habitat, astomatous leaves covered by a thick cuticle, an elongate stem and large hollow unbranched roots. It is quite distinct in that it occurs only as separate rosettes that are many times larger than those of *I. andicola* (Table 9.8). In contrast to *I. andicola*, the triquetrous-shaped, spine-tipped leaves represent a much larger proportion of the biomass (Table 9.8). As with *I. andicola*, only the upper third of *I. andina* leaves is chlorophyllous, but typically the entire plant is sunk into the peat, often below ground level (J. Keeley, unpublished data). *Isoetes andina* grows in habitats similar to those of *I. andicola*, although the highly

Table 9.8 Dimensions and mass of terrestrial Isoetes and associated rosette forming species from Chisaca site, Colombia

See Appendix 9.1 for precise locality. Methods are described in the text.

	<i>Isoetes andina</i>	<i>Oreobolus obtusangulus</i>	<i>Plantago rigida</i>
Leaves			
Stomata			
% gas space	Absent	Abundant	Abundant
% of total biomass	27	28	8
Roots	65	83	41
Branching			
% gas space	Absent	Extensive	Extensive
	46	10	35
Rosette areal coverage (cm ² per rosette) ^a	115.7 ± 46.3	1.8 ^a ± 1.2	3.8 ^a ± 1.9
Leaves per rosette	195.9 ± 71.3	17.4 ^a ± 6.3	29.9 ^a ± 14.6
Leaf production			
(g ODW per rosette per season)	2.865 ± 0.717	0.017 ^a ± 0.003	0.062 ^a ± 0.029
(g ODW cm ⁻² per season)	0.025 ± 0.006	0.009 ± 0.002	0.016 ± 0.007
Fresh weight	8.90	8.00	4.90
Dry weight			

^a $\bar{X} \pm \text{SD}$, $n = 10$ (values within a row with the same superscript are not significantly different at $p > 0.05$, with 2-tailed t -test).

organic sediment is not quite as rich in CO₂ as the peat sediment of *I. andicola* (Table 9.4).

Preliminary laboratory measurements with *I. andina* indicate similarities with *I. andicola* in the form of carbon nutrition; one experiment similar to those presented in Table 9.3 showed four times greater CO₂ fixation by green leaves when CO₂ was fed to roots than when fed to leaves (10.3 vs 2.7 $\mu\text{mol CO}_2 \text{ mg}^{-1} \text{ Chl h}^{-1}$).

In the páramo of Ecuador is another terrestrial *Isoetes*, *I. novo-granadensis* Fuchs, which probably has a similar mode of carbon nutrition. This is suggested by its astomatous leaves with thick cuticle and its habit of being buried in the sediment, similar to *I. andina*. Both of these taxa, however, have substantially less intercellular gas space in the leaves and roots (Tables 9.8, 9.9) than *I. andicola*. This may reflect less of a dependence upon diffusion of carbon dioxide from the sediment than has been demonstrated for *I. andicola*, although it may also reflect structural constraints imposed by the larger rosettes of *I. andina* and *I. novo-granadensis*. Additionally, there is evidence that both of these species have Crassulacean Acid Metabolism (Appendix 9.1).

As with *I. andicola*, there is some degree of convergence in the rosette growth habit of species associated with *I. andina* and *I. novo-granadensis*. Characteristics of the important species associated with each are presented in Tables 9.8 and 9.9. The presence of stomata in these other taxa suggests active gas exchange with the aerial atmosphere and there is no evidence of CAM (Appendix 9.1). Only one of these four species with typical CO₂ uptake from the atmosphere had greater seasonal leaf production than the associated *Isoetes* species (Tables 9.8, 9.9).

The syndrome of sediment-based carbon nutrition in terrestrial plants is apparently restricted to species of *Isoetes* in tropical alpine habitats; however, it is apparently not restricted to South America. The recently described *I. hopei* Croft from the tropical alpine parts of western New Guinea is in all likelihood an example. Based on photographs it would be difficult to distinguish it from *I. andina* and the published description (Croft 1980) suggests a similar habit; *I. hopei* is a terrestrial species with triquetrous leathery leaves lacking stomata.

Adaptive significance of sediment-based nutrition in tropical alpine habitats

Because of decreased barometric pressure, the partial pressure of carbon dioxide declines with increasing altitude. Therefore, one might predict a

Table 9.9 Dimensions and mass of terrestrial Isoetes and associated rosette forming species from Quito-Baeza site, Ecuador

See Appendix 9.1 for precise locality. Methods are described in the text.

	<i>Isoetes novo-granadensis</i>	<i>Wernaria humilis</i>	<i>Oritrophium peruvianum</i>
Leaves			
Stomata			
% gas space	Absent	Abundant	Abundant
% of total biomass	34	25	38
	45	43	14
Roots			
Branching			
% gas space	Absent	Present	Very little
	39	29	35
Rosette areal coverage (cm ² per rosette)	33.8 ± 17.8	13.4 ± 3.7	114.2 ± 55.5
Leaves per rosette	53.4 ± 40.0	116.2 ± 29.2	32.4 ± 7.4
Leaf production			
(g ODW per rosette per season)	1.100 ^a ± 1.150	0.910 ^a ± 0.313	0.100 ^a ± 0.048
(g ODW cm ⁻² per season)	0.032 ± 0.014	0.069 ± 0.022	0.014 ± 0.006
Fresh weight	9.20	4.61	4.33
Dry weight			

^a $\bar{X} \pm \text{SD}$, $n = 10$ (values within a row with the same superscript are not significantly different at $p > 0.05$, with 2-tailed t -test).

selective advantage to plants utilizing a decomposing peat sediment, which is enriched in carbon dioxide, as a photosynthetic source. In support of this idea, Billings & Godfrey (1967) showed that the photosynthetic rate of the temperate-alpine *Mertensia ciliata* was significantly reduced when the carbon dioxide concentration was reduced to levels approximating those at an altitude of 3100 m. They suggested this as a factor in the utilization of respiratory carbon dioxide which accumulated within the hollow stems of this plant. However, such analysis is complicated by the increased diffusion coefficient of CO₂ with a drop in barometric pressure. Calculations suggest that the increased rate of diffusion of CO₂ with altitude largely offsets the inhibitory effects of decreased partial pressure of this gas at high altitude (Gale 1972).

All tropical alpine terrestrial *Isoetes* are distributed near lakes or lagoons, although all are distributed well above the water level and, due to seasonal droughts (e.g. Table 9.1), may be subjected to soil water stress at certain times during the season. By sealing the leaves from the atmosphere this could provide an advantage under water stress conditions. In other words, if soil water stress is sufficient to cause stomatal closure in stomatous species, then a plant that obtains carbon from the sediment may be able to continue photosynthesis at times when other species are carbon-limited. At the present, evidence in support of this hypothesis is largely lacking. Most of the species studied here have leaves too small for conventional porometry. However, preliminary studies during the dry season at Junin, Peru, did show differences in midday water potentials between *Isoetes andicola* and associated species that would be consistent with this hypothesis. On 26 July 1986, midday water potentials (as measured with a Schollander-type pressure chamber, $n = 3$) were -2.63 ± 0.50 MPa for *Plantago rigida*, -2.02 ± 0.43 for *Distichia muscoides*, and -0.60 ± 0.30 for *Isoetes andicola*. These patterns were repeated on two other dates during the same month.

Evolution of sediment-based nutrition in terrestrial *Isoetes*

We hypothesize that the evolution of sediment-based carbon nutrition in terrestrial *Isoetes* taxa of tropical alpine habitats is the result of a pre-adaptation present in aquatic ancestors. Cladistic analyses (Hickey 1985) suggests an aquatic ancestry and there is reason to believe such ancestral taxa obtained carbon from the sediment.

Aquatic *Isoetes* are nearly ubiquitous in the tropical alpine region of South America and thus potential ancestral aquatic taxa are found

throughout the range of Neotropical terrestrial *Isoetes*. Indeed, one to several aquatic species are present in lakes adjacent to all terrestrial *Isoetes* sites studied here. Although there is no experimental evidence that these aquatic species obtain carbon from the organic-rich sediment, there is documentation that Northern Hemisphere *Isoetes* species obtain the bulk of their carbon from the sediment (Richardson *et al.* 1984; Boston *et al.* 1987). These Northern Hemisphere *Isoetes* taxa are distributed in oligotrophic lakes that share some features with Neotropical alpine lakes. Particularly relevant is the differential between water column and sediment CO₂ concentrations; for example, a small lake near Laguna Chisaca, Colombia had a water column [CO₂] = 0.26 mol m⁻³, whereas the sediment [CO₂] = 1.70 mol m⁻³ (J. Keeley, unpublished data). Collections of aquatic *Isoetes* from Colombia, Venezuela, Ecuador, Peru and Bolivia have revealed a pattern of morphological and anatomical characteristics consistent with this hypothesized mode of carbon nutrition. All taxa lack stomata, which is to be expected in aquatic plants, but more importantly, all taxa have a very thick cuticle covering the leaf surface, which is not expected in aquatic plants, except in plants obtaining carbon from the sediment where a thick leaf cuticle would prevent the leakage of CO₂ into the water column. These aquatic *Isoetes* also have leaves that are buried up to two thirds of their length in the sediment and they have massive roots, often extending to more than 50 cm depth. Additionally, leaves, stems and roots of all of these aquatic *Isoetes* have extensive intercellular gas space (commonly the percentage of cross-sectional gas space is greater than recorded here for *I. andicola*). All of these aquatic species also possess a very well developed CAM pathway (J. Keeley, unpublished data).

If terrestrial taxa did evolve from aquatic taxa, cladistic analysis suggests that this event happened more than once. Based on a study of morphological characters, Hickey (1985) concluded that the three terrestrial taxa discussed here were derived from separate lineages. There is some chemical evidence to support this decision. In a preliminary survey of flavonoid patterns it was found that the terrestrial species, *I. andina* of Colombia and *I. novo-granadensis* of Ecuador produced chromatographic spot patterns more similar to nearby aquatic taxa than to each other or to *I. andicola* of Peru (Figure 9.6). The spot patterns produced by *I. andicola* were not obviously related to any of the dozen taxa investigated, which is consistent with Hickey's (1985) conclusion that this taxon is quite distant from other *Isoetes*.

Isoetes andina (No. 7902, collection no. of senior author) produced a chromatographic pattern very close to the aquatic *I. cleefii* Fuchs (No.

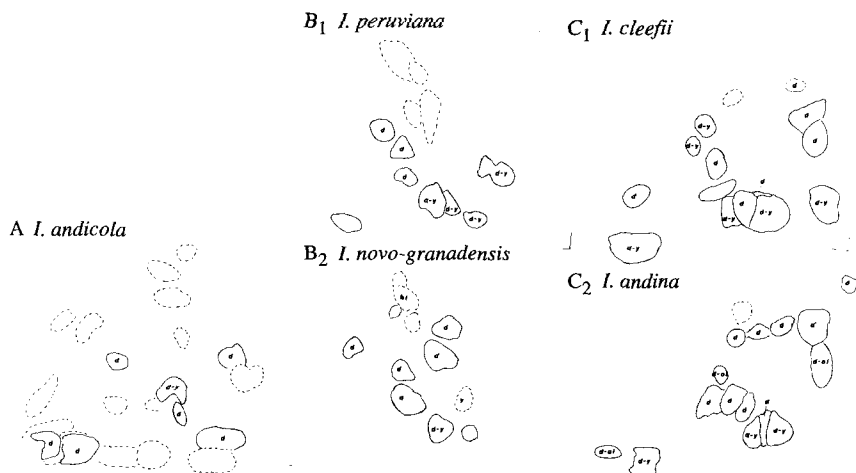


Figure 9.6. Chromatographic spot patterns for (A) terrestrial *Isoetes andicola* from Peru; (B₁) aquatic *I. cleefii* and (B₂) terrestrial *I. andina* from Colombia; (C₁) aquatic *I. peruviana* and (C₂) terrestrial *I. novo-granadensis* from Ecuador. Solid outlines indicate a flavonoid pigment appearing in UV (360 nm) and dotted outlines indicate a non-flavonoid compound that fluoresces in UV light (d, dark; y, yellow; ol, olive; bl, blue; d-y, dark changed to yellow). Origin is in the right-hand corner; vertical separation was with 15% HOAc and the horizontal axes were separated with TBA.

7876) collected from a nearby site (Figure 9.6, B) and similar to *I. karstenii* A. Braun (Nos. 7865, 7909, 10082) from a lake adjacent to the *I. andina* site. Based on morphology, these taxa are considered to be closely aligned; in Hickey's (1985) treatment, *I. cleefii* and *I. karstenii* are included in the *I. andina* alliance.

The chromatographic spot patterns generated by the terrestrial *I. novo-granadensis* (No. 10014) were quite unlike terrestrial *I. andina* but strikingly similar to the aquatic *I. peruviana* (No. 10020) Weber collected from a pond adjacent to the *I. novo-granadensis* site (Figure 9.6, C) and also very similar to another aquatic *I. killipii* (No. 10025) from a nearby lake. These taxa are also closely aligned by Hickey (1985 and personal communication).

Although the reasons are not clear, it appears that correlated with the evolution of this terrestrial syndrome is an increase in ploidy level. The common condition in the genus is $2n = 22$. *Isoetes andicola* has $2n = 44$. The aquatic *I. karstenii* has $2n = 22$ whereas the related terrestrial *I. andina* has $2n = 66$ (P. Hickey and J. Keeley, unpublished data). For *I. novo-granadensis*, $2n = 132$ (Hickey 1984) and the closest aquatic

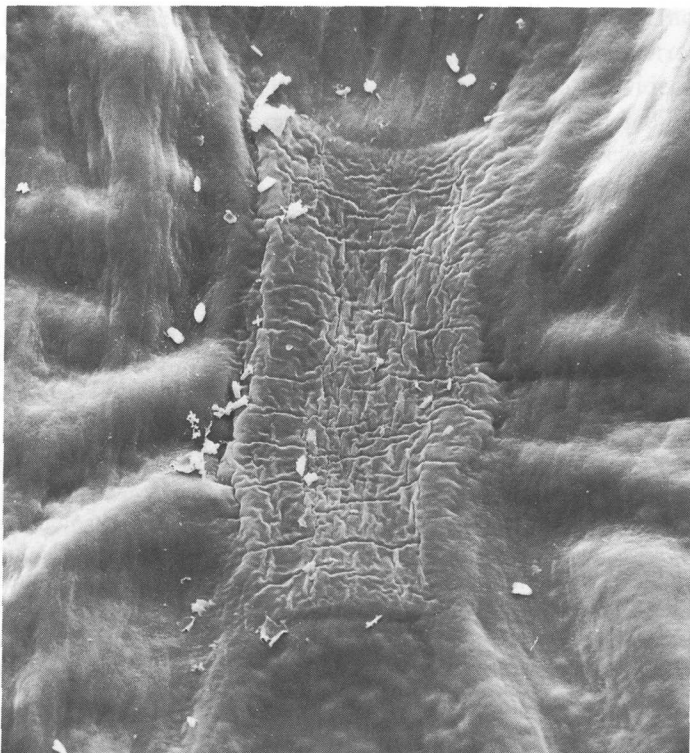


Figure 9.7. SEM (2000 \times) of *Isoetes andicola* leaf surface showing odd cell interpreted as an aborted guard mother cell.

relatives, *I. killipii* and *I. peruviana*, have $2n = 66$ and 44, respectively (R. Hickey and J. Keeley, unpublished data).

One observation, which could be interpreted as inconsistent with this evolutionary scenario, is the presence of a very unusual cell on the surface of *Isoetes andicola* (Figure 9.7). These cells are regularly distributed in linear arrays, similar to the arrangement of stomata in *Isoetes* taxa that possess stomata. One interpretation of these structures is that they are guard mother cells that aborted during development, which, if correct, would suggest that an ancestor of *I. andicola* had functional stomata. In Neotropical *Isoetes* stomata are present in amphibious taxa that occur in seasonally inundated habitats. If such a taxon were part of *I. andicola*'s ancestral heritage, it suggests strong selection against stomatal production in the present environment. These unusual cells (Figure 9.7), however, are

not present in *I. andina* or *I. novo-granadensis* and thus evolution on to land may have followed more than one course.

Acknowledgements

The senior author is indebted to James Teeri, who provided the airline ticket that allowed for the initial visit to Peru for work with *Stylites*, and Sterling Keeley for field assistance. John Raven and Barry Osmond have made many intellectual contributions. This work has also been supported by NSF grants TFI-8100529, DEB-8004614, DEB-82-06887 and BSR-8407935, National Geographic Society grant No. 3119-15, and a fellowship from the John Simon Guggenheim Foundation to the senior author. Irwin Ting and Loretta Bates kindly provided the dual isotope porometer data on *Stylites* and Dan Walker and Judy Verveke provided the SEM.

References

- Amstutz, E. (1957). *Stylites*, a new genus of Isoetaceae. *Annals of the Missouri Botanical Garden* **44**, 121-3.
- Billings, W. D. & Godfrey P. J. (1967). Photosynthetic utilization of internal carbon dioxide by hollow-stemmed plants. *Science* **158**, 121-3.
- Boston, H. L. & Adams, M. S. (1985). Seasonal diurnal acid rhythms in two aquatic crassulacean acid metabolism plants. *Oecologia* **65**, 573-9.
- Boston, H. L., Adams, M. S. & Pienkowski, T. P. (1987). The utilization of sediment CO₂ by selected North American isoetids. *Annals of Botany* **60**, 485-94.
- Cleef, A. M. (1981). *The Vegetation of the Páramos of the Colombian Cordillera Oriental*. Hirschberg: J. Cramer.
- Croft, J. R. (1980). A taxonomic revision of *Isoetes* L. (Isoetaceae) in Papuasias. *Blumea* **26**, 177-90.
- Dacey, J. W. H. (1980). Internal winds in waterlilies: an adaptation for life in anaerobic sediments. *Science* **210**, 1017-19.
- Fuchs-Eckert, H. P. (1982). Zur heutigen Kenntnis von Vorkommen und Verbreitung der sudamerikanischen *Isoetes*-Arten. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C* **85**, 205-60.
- Gale, J. (1972). Availability of carbon dioxide for photosynthesis at high altitudes: theoretical considerations. *Ecology* **53**, 494-7.
- Gomez, L. D. (1980). Vegetative reproduction in a Central American *Isoetes* (Isoetaceae). Its morphological, systematic and taxonomical significance. *Brenesia* **18**, 1-14.
- Hickey, R. J. (1984). Chromosome numbers of Neotropical *Isoetes*. *American Fern Journal* **74**, 9-13.
- Hickey, R. J. (1985). Revisionary studies of Neotropical *Isoetes*. PhD dissertation, University of Connecticut, Storrs.

- Karrfalt, E. E. & Hunter, D. M. (1980). Notes on the natural history of *Stylites gemmiflora*. *American Fern Journal* **70**, 69–72.
- Keeley, J. E. (1981). *Isoetes howellii*: A submerged aquatic CAM plant? *American Journal of Botany* **68**, 420–4.
- Keeley, J. E. (1982). Distribution of diurnal acid metabolism in the genus *Isoetes*. *American Journal of Botany* **69**, 254–7.
- Keeley, J. E. (1987). The adaptive radiation of photosynthetic modes in the genus *Isoetes* (Isoetaceae). In *Plant Life in Aquatic and Amphibious Habitats*, ed. R. M. M. Crawford, pp. 113–28. Oxford: Blackwell Scientific Publications.
- Keeley, J. E. & Busch, G. (1985). Carbon assimilation characteristics of the aquatic CAM plant, *Isoetes howellii*. *Plant Physiology* **76**, 525–30.
- Keeley, J. E. & Keeley, S. C. (1989). Crassulacean acid metabolism (CAM) in high elevation tropical cactus. *Plant, Cell and Environment* **12**, 331–6.
- Keeley, J. E., Osmond, C. B. & Raven, J. A. (1984). *Stylites*, a vascular land plant without stomata absorbs CO₂ via its roots. *Nature* **310**, 694–5.
- Keeley, J. E., Sternberg, L. O. & DeNiro, M. J. (1987). The use of stable isotopes in the study of photosynthesis in freshwater plants. *Aquatic Botany* **26**, 213–23.
- Kluge, M. & Ting, I. P. (1978). *Crassulacean Acid Metabolism*. New York: Springer-Verlag.
- Kubitzki, K. & Borchert, R. (1964). Morphologische Studien an *Isoetes triquetra* A. Braun und Bemerkungen über das Verhältnis der Gattung *Stylites* E. Amstutz zur Gattung *Isoetes* L. *Berichte der Deutschen Botanischen Gesellschaft* **77**, 227–33.
- Mooney, H. A., Harrison, A. T. & Gulmon, S. L. (1974). Photobleaching in high and low elevation plants and different radiation intensities. *American Midland Naturalist* **91**, 254–6.
- Nobel, P. S. (1983). *Biophysical Plant Physiology and Ecology*. San Francisco: W. H. Freeman.
- Rauh, W. & Falk, H. (1959). *Stylites* E. Amstutz, eine neue Isoetaceae aus den hochandern Perus. 1. Teil: Morphologie, anatomie und entwicklungsgeschichte der vegetationsorgane. *Sitzungsberichte der Heidelberger Akademie der Wissenschaften, Mathematischen-naturwissenschaftliche Klasse* **1959** **2**, 85–160.
- Richardson, K., Griffiths, H., Reed, M. L., Raven, J. A. & Griffiths, N. M. (1984). Inorganic carbon assimilation in the isoetids, *Isoetes lacustris* L. and *Löbelia dortmanna* L. *Oecologia* **61**, 115–21.
- Sondergaard, M. & Sand-Jensen, K. (1979). Carbon uptake by leaves and roots of *Littorella uniflora* (L.) Aschers. *Aquatic Botany* **6**, 1–12.
- Sorrell, B. K. & Dromgoole, F. I. (1987). Oxygen transport in the submerged freshwater macrophyte *Egeria densa* Planch. I. Oxygen production, storage and release. *Aquatic Botany* **28**, 63–80.
- Sternberg, L. & DeNiro, M. J. (1983). Isotopic composition of cellulose from C₃, C₄, and CAM plants growing in the vicinity of one another. *Science* **220**, 947–8.
- Sternberg, L., DeNiro, M. J. & Keeley, J. E. (1984). Hydrogen, oxygen, and carbon isotope ratios of cellulose from submerged aquatic crassulacean acid metabolism and non-crassulacean acid metabolism plants. *Plant Physiology* **76**, 69–70.

- Sternberg, da S. L., DeNiro, M. J., McJunkin, D., Berger, R. & Keeley, J. E. (1985). Carbon, oxygen and hydrogen isotope abundances in *Stylites* reflect its unique physiology. *Oecologia* **67**, 598–600.
- Tryon, R. M. & Tryon, A. F. (1982). *Ferns and Allied Plants*. New York: Springer-Verlag.
- Wium-Andersen, S. (1971). Photosynthetic uptake of free CO₂ by the roots of *Lobelia dortmanna*. *Physiologia Plantarum* **25**, 245–8.
- Ziegler, H., Osmond, C. B., Stickler, W. & Trimborn, D. (1976). Hydrogen isotope discrimination in higher plants: correlation with photosynthetic pathway and environment. *Planta* **128**, 85–92.

Appendix 9.1 Morning and evening levels of titratable acidity (to pH 7.0 or, if indicated with an asterisk, to pH 6.4) and malic acid in photosynthetic tissues of tropical alpine terrestrial Isoetes and associated species (vouchers deposited in LOC)

Species	Date	Country ^b	Titratable acidity ($\mu\text{mol H}^+ \text{ g}^{-1} \text{ FW}$)		Malic acid ($\mu\text{mol g}^{-1} \text{ FW}$)	
			p.m. $\bar{X} \pm \text{SD}^a$	a.m. $\bar{X} \pm \text{SD}$	p.m. $\bar{X} \pm \text{SD}$	a.m. $\bar{X} \pm \text{SD}$
BRYOPHYTA						
(Sphagnaceae)						
<i>Sphagnum</i> sp.						
	Dec	Colo.	50* \pm 6	58* \pm 2	1 \pm 2	1 \pm 1
	Jul	Colo.	65 \pm 3	35 \pm 7	15 \pm 6	14 \pm 5
TRACHEOPHYTA						
Lycopsida						
(Isoetaceae)						
<i>Isoetes (Stylites) andicola</i> (Amstutz) Gomez						
	Nov	Peru	60 \pm 10	150 \pm 30	15 \pm 5	55 \pm 15
	Jul	Peru	81 \pm 7	180 \pm 31	23 \pm 6	69 \pm 16
<i>I. andina</i> Hook						
	Dec	Colo.	37* \pm 3	72* \pm 11	40 \pm 11	59 \pm 3
	Jul	Colo.	22 \pm 3	204 \pm 42	32 \pm 9	77 \pm 19
<i>I. novo-granadensis</i> Fuchs						
	Nov	Ecua.	3* \pm 4	132* \pm 31	41 \pm 16	82 \pm 6
	Jul	Ecua.	59 \pm 34	201 \pm 7		
Spermopsida – Monocotyledoneae						
(Juncaceae)						
<i>Distichia muscoides</i> Nees et Meyen						
	Nov	Peru	6 \pm 0	3 \pm 0		
(Cyperaceae)						
<i>Oreobolus obtusangulus</i> Gaud.						
	Nov	Ecua.	0* \pm 0	0* \pm 0	15 \pm 8	17 \pm 5

Appendix 9.1 (continued)

Species	Date	Country ^b	Titratable acidity ($\mu\text{mol H}^+ \text{ g}^{-1} \text{ FW}$)		Malic acid ($\mu\text{mol g}^{-1} \text{ FW}$)	
			p.m. $\bar{X} \pm \text{SD}^a$	a.m. $\bar{X} \pm \text{SD}$	p.m. $\bar{X} \pm \text{SD}$	a.m. $\bar{X} \pm \text{SD}$
(Eriocaulaceae)						
<i>Paepalanthus karsgenii</i> Ruhl.						
	Jul	Colo.	28 \pm 4	30 \pm 2	3 \pm 1	8 \pm 7
Spermopsida – Dicotyledoneae						
(Apiaceae)						
<i>Eryngium humile</i> Cav.						
	Jul	Colo.	18 \pm 5	26 \pm 2	17 \pm 4	26 \pm 9
(Asteraceae)						
<i>Espeletia grandiflora</i> Humb. et Bonpl.						
	Jul	Colo.	95 \pm 7	73 \pm 11	21 \pm 4	4 \pm 6
<i>Oritrophium peruvianum</i> (Lam.) Cuatr.						
	Nov	Ecu.	9 \pm 4	8 \pm 4		
<i>Wernaria humilis</i> H.B.K.						
	Nov	Ecu.	0* \pm 0	0* \pm 0	2 \pm 3	15 \pm 9
	Jul	Ecu.	15 \pm 2	14 \pm 3	3 \pm 5	0 \pm 0
(Plantaginaceae)						
<i>Plantago rigida</i> H.B.K.						
	Nov	Ecu.	0* \pm 0	0* \pm 0	14 \pm 2	25 \pm 4
	Jul	Peru	38 \pm 5	38 \pm 4	41 \pm 11	63 \pm 19
	Jul	Colo.	45 \pm 1	42 \pm 3	39 \pm 16	15 \pm 1

^a Mean \pm standard deviation ($n = 3$).

^b Sites were as follows.

Peru: Mounds of peat deposit, 60–125 cm elevation above surrounding bog, 4180 m; 100 m W of Route 3, 19.1–19.3 km NW of Junin town limit, Departamento de Junin (11° 00' S; 76° 00' W).

Ecuador: Boggy area, 65–390 cm elevation above shallow pool, 4040 m; below antennas at pass N of road from Quito to Baeza, 46.6 km (on old road) E of Plaza Frederico Pizarro in Quito, Provincia del Napo (00° 20' S; 78° 10' W).

Colombia: Heavy moss cover, 50–205 cm elevation above Laguna Chisaca, 3650 m; Páramo de Sumapaz, 30 km S of Usme, Departamento de Cundinamarca (4° 14' S; 74° 16' W).

TROPICAL ALPINE ENVIRONMENTS

Plant form and function

Edited by

PHILIP W. RUNDEL

Department of Biology, University of California, Los Angeles

ALAN P. SMITH

Formerly of Smithsonian Tropical Research Institute, Panama City, Panama

F. C. MEINZER

Hawaiian Sugar Planters Association, Aiea, Hawaii



CAMBRIDGE
UNIVERSITY PRESS

1994